

Technical Explanation

US Application 10/583,706

Dr.Yoshihide Hayashizaki
11.08.2010

Claim 1 of the present application

(TP-FP primer set)

A primer set comprising at least two primers that allows a target nucleic acid sequence to be amplified,

wherein a **first primer** included in the primer set contains, in its 3' end portion, a sequence (Ac') that hybridizes to a sequence (A) located in the 3' end portion of the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Ac'), a sequence (B') that hybridizes to a complementary sequence (Bc) to a sequence (B) that is present on the 5' side with respect to the sequence (A) in the target nucleic acid sequence, and

a **second primer** included in the primer set contains, in its 3' end portion, a sequence (Cc') that hybridizes to a sequence (C) located in the 3' end portion of a complementary sequence to the target nucleic acid sequence, and also contains, on the 5' side of the sequence (Cc'), a folded sequence (D-Dc') that contains, on the same strand, two nucleic acid sequences that hybridize to each other.

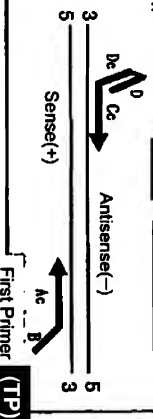
*The first primer is **TP**, the second primer is **FP**.

TP; Turn-back Primer

FP; Folded Primer

Technical explanation of the TP and FP

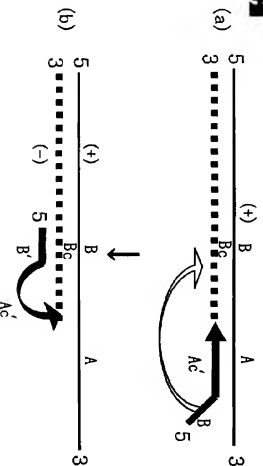
Second Primer (FP) Primer Set



TP has the function as follows;

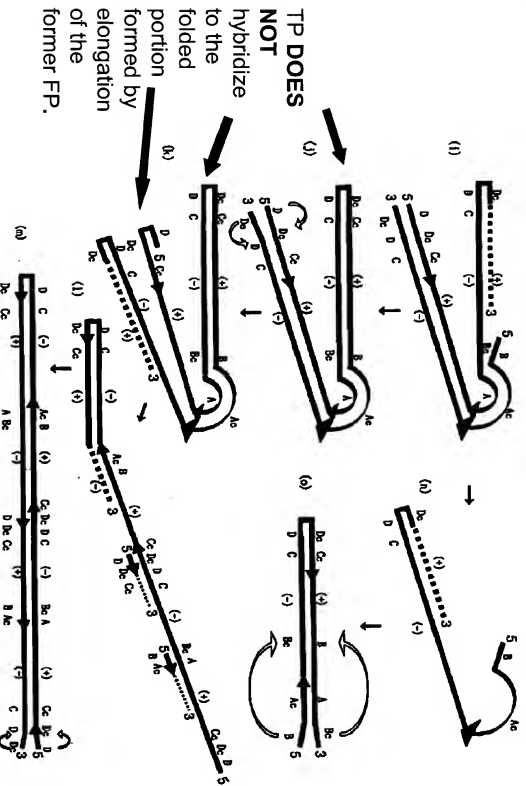
- (1) TP has the turn back portion (B) in the 5' side sequence.
- (2) The turn back portion (B) can hybridize to the portion (Bc) of the elongation strand from TP.

- FP has the function as follows;**
- (1) FP has the folded sequence (D- Dc') in the 5' side sequence.
 - (2) The folded sequence (D-Dc') has two nucleic acid sequences that hybridize to each other.
 - (3) The folded sequence (D-Dc') **DO NOT** hybridize to the elongation strand from FP.



Mechanism of the amplification reaction of the TP-FP(2)

(FIG.3 of the present invention)



The present invention has four advantages.

(1) Isothermal amplification

- The amplification occurs without thermal denaturation.

(2) Specific amplification

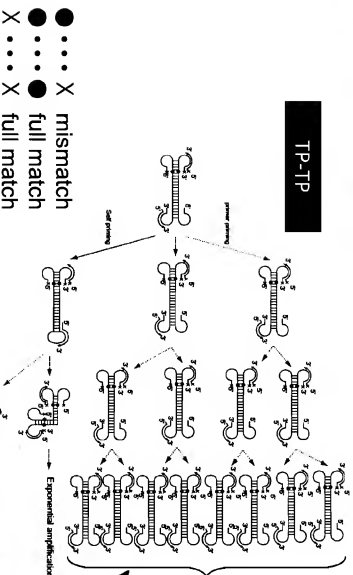
- The present invention can detect SNPs without non-specific amplification.

(3) Short time amplification

(4) Easy primer design

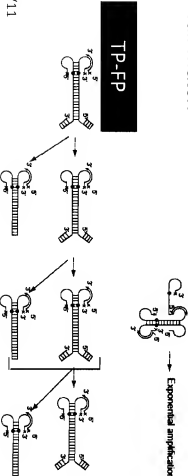
Mechanism of specific amplification

TP-TP



- (1) TP-TP primer set CAN NOT detect SNPs.
- (2) Because, TP-TP primer set has the pathway of the non-specific extensively exponential amplification (background amplification).

Exponential amplification



- (1) TP-FP primer set **CAN** detect SNPs.
- (2) Because, the non-specific exponential amplification of the pathway of TP-FP primer set is very gentle.

Short time amplification and easy primer design(1)

(1) TP

- (i) TP can amplify exponentially.
- (ii) TP has a strong engine of amplification.
- (iii) TP has two area depending on template sequence.

(2) FP

- (i) FP can not amplify exponentially, but amplify linearly.
- (ii) FP is like a mirror which reflect TP amplification.
- (iii) FP needs only one area depending on template sequence.

(3) TP-TP primer set

- (i) TP-TP primer set needs four areas depending on the template sequence.
- (ii) TP-TP Primer set needs a design of a couple of good TP because the reaction is totally controlled by no good TP.
- (iii) TP-TP Primer set is difficult to design.

(4) TP-FP primer set

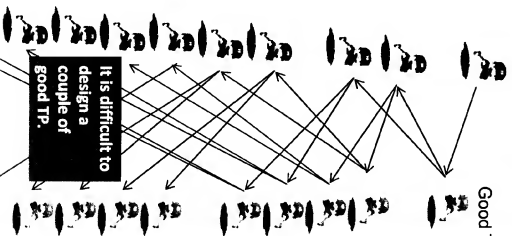
- (i) TP-FP primer set needs only three areas depending on the template sequence.
- (ii) TP-FP Primer set needs a design of only one good TP because FP whose folded sequence can be designed in advance independently from template sequence **DOES NOT** control the reaction.
- (iii) TP-FP Primer set is easy to design.

Short time amplification and easy primer design(2)

TP+TP

Good TP

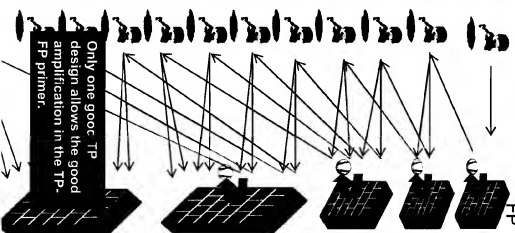
Good TP



TP+FP

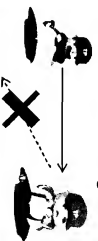
Good TP

FP



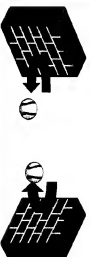
Good TP

No good TP



The no good TP controls the amplification reaction.

FP only reflects the TP amplification.



Office Action (1)

• Summary of the Office Action

The examiner pointed out as follows;

- (1) TP are shown in Figure 4, step 1 and 2 (① and ② shown in below left) in Rabbani (EP0971039A2).
- (2) FP are shown in Figure 1, step 3 (③ shown in below right) in Rabbani.
- (3) Therefore, Claims 1 to 5 of the present invention lacks novelty (102(b)).

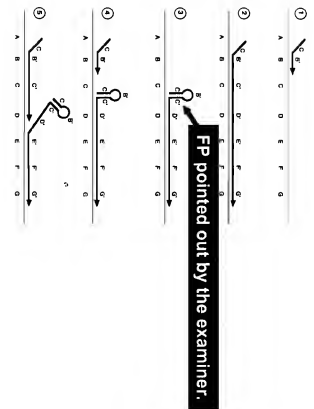
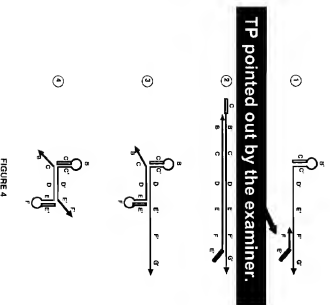
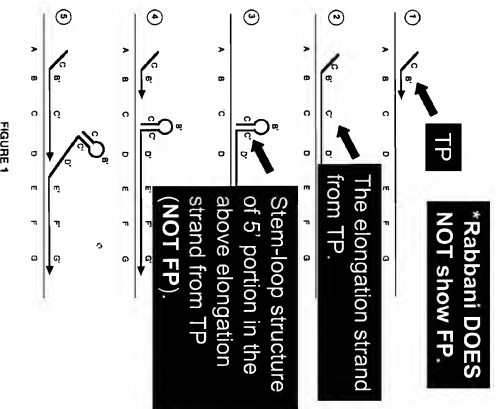
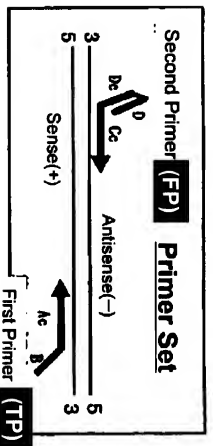


FIGURE 4

FIGURE 1

Office Action (2); FIGURE 1 ③ in Rabbani is NOT FP.

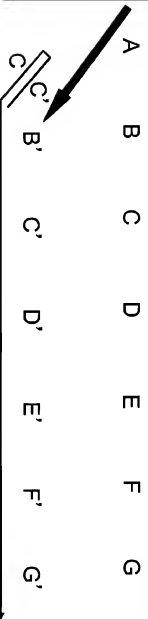
- (1) FIGURE 1 ③ in Rabbani shows the elongation strand from TP.
- (2) **Primer is different from the elongation strand.**
- (3) Rabbani **DOES NOT** show the TP-FP primer set of the present invention.



In case FP is used in the FIGURE 1 in Rabbani.

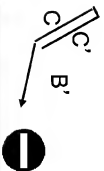


The sequence B'
DOES NOT form
stem-loop
structure (B
DOES NOT form
a single strand).



A B C D E F G

Stem-loop structure is not formed (Turn
back reaction **DOES NOT** occur).



New primer **DOES**
NOT hybridizes to
the sequence B in
the template
nucleic acid.



A B C D E F G